Royal Government of Bhutan
Ministry of Health
Department of Public Health
Zoonotic Disease Program

National Guideline for Management of Rabies


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## ACRONYMS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>ARV</td>
<td>Anti Rabies Vaccine</td>
</tr>
<tr>
<td>DPT</td>
<td>Diphtheria, Pertussis and Tetanus</td>
</tr>
<tr>
<td>DRA</td>
<td>Drug Regulatory Authority</td>
</tr>
<tr>
<td>DoL</td>
<td>Department of Livestock</td>
</tr>
<tr>
<td>DVH</td>
<td>Dzongkhag Veterinary Hospital</td>
</tr>
<tr>
<td>HRIG</td>
<td>Human Rabies Immunoglobulin</td>
</tr>
<tr>
<td>HDCV</td>
<td>Human Diploid Cell Vaccine</td>
</tr>
<tr>
<td>IM</td>
<td>Intramuscular</td>
</tr>
<tr>
<td>ID</td>
<td>Intra Dermal</td>
</tr>
<tr>
<td>LEC</td>
<td>Livestock Extension Center</td>
</tr>
<tr>
<td>MoAF</td>
<td>Ministry of Agriculture and Forests</td>
</tr>
<tr>
<td>NCAH</td>
<td>National Centre for Animal Health</td>
</tr>
<tr>
<td>PCECV</td>
<td>Purified chick-embryo cell-culture vaccine</td>
</tr>
<tr>
<td>PVRV</td>
<td>Purified Vero Cell Rabies Vaccine</td>
</tr>
<tr>
<td>PrEP</td>
<td>Pre-exposure prophylaxis</td>
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<tr>
<td>PEP</td>
<td>Post Exposure Prophylaxis</td>
</tr>
<tr>
<td>PHL</td>
<td>Public Health Laboratory</td>
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<tr>
<td>RH</td>
<td>Regional Hospitals</td>
</tr>
<tr>
<td>RIG</td>
<td>Rabies immunoglobulin</td>
</tr>
<tr>
<td>TT</td>
<td>Tetanus Toxoid</td>
</tr>
<tr>
<td>Td</td>
<td>Tetanus Toxiod</td>
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Background

Rabies is a fatal zoonotic disease caused by a RNA virus. After entering the central nervous system of the host, the virus causes an acute progressive encephalomyelitis and death within a few days. Rabies is transmitted from animal to animal or from animal to human. Human infection by rabies virus usually occurs as a result of transdermal bite, lick or scratch from an infected animal.

Although rabies is a fatal and incurable disease, it is still neglected in many developing countries. Unlike other communicable diseases, rabies and death is preventable by post exposure prophylaxis with anti rabies vaccine. However, rabies remains a major public health problem, especially in the developing world.

Global Situation

Although rabies is 100% preventable, worldwide this fatal disease still claims over 55,000 human lives annually. Dog bite is responsible for more than 95% of human rabies infection in endemic countries where stray dogs outnumber pet dogs and dog vaccination against rabies is not mandatory. Most of the victims are children and most of the deaths occur in Asia and Africa. These deaths could be easily prevented if proper post-exposure rabies prophylaxis was made available to the exposed.

In recent years many developed countries have completely eliminated rabies and other countries have reduced its incidence to a minimum level. These include some Asian countries (Malaysia and Japan) that have controlled the disease through implementation of strict control measures.

Country Situation

Rabies is a priority zoonotic disease of concern for both the human and animal health sectors in Bhutan and is a notifiable disease in Bhutan. Rabies commonly occurs in the southern belt of Bhutan along the borders with India; however, isolated cases have been documented in the northern parts of the country, especially in migratory domestic animals (Figure 1). Fifty-nine of the 205 geog (sub-districts) reported rabies in animals from 1996 to 2013 with increased incidences in the four districts in southern Bhutan (Samtse, Chukha, Sarpang, Samdrup Jongkhar). Between 1996 and 2013, 939 rabies cases were reported in dogs (375, 40%), cattle (475, 52%) and other domestic animals (cats, goats, pigs, sheep: 70, 8%) with an average of 17 outbreaks reported every year (Figure 2). Rabies outbreaks in animals were found to have been reported throughout the year with more reports during spring and summer months (Figure 3).
Figure 1: Distribution of reported rabies outbreaks in animals at Dzongkhag level & the number of reported rabies cases in dogs and other domestic animals (*within bracket) in Bhutan (Jan 1996 - Dec 2013). Outbreak in Paro occurred in 1999 while outbreaks in eastern Bhutan (Trashiyangtse, Trashigang & Mongar) occurred between 2005 and 2007.

Figure 2: Annual pattern of reported rabies outbreaks in animals in Bhutan at the village level (1996 – 2013).
Figure 3: Monthly distribution of reported rabies outbreaks and cases in animals (dogs and other domestic animals) in Bhutan (1996 – 2013).

Sporadic human deaths from rabies are also reported in the south rabies endemic Dzongkhags of Bhutan. For instance, 21 human deaths (mostly children) were reported between 2006 and 2013 (2006: 3 deaths; 2007: 3; 2008: 2; 2009: 4; 2010: 3; 2011: 5; 2012: 0 and 2013: 1 death).

**Causative Agent**

The rabies virus is a single stranded, enveloped RNA virus, belonging to the genus Lyssavirus and family Rhabdoviridae. Rabies virus is neurotropic and widely distributed in the nervous systems, saliva and all secretions of infected animals. The virus occurs in highest concentration in the nervous system and in the salivary glands. Rabies virus is fragile and easily inactivated by sunlight, heat and the commonly used disinfectants.

**Reservoir of Infection**

Rabies exists in two epidemiological forms:

1. **Urban Rabies**

The stray dog population in the country maintains the urban cycle. This cycle is responsible for 99% of human infections. Although rare, other domestic animals such as cats, cattle, sheep, goat, horse and pigs can also transmit the disease through bites or consumption of infected raw meat by people.
2. **Sylvatic or Wild life Rabies**

Mainly perpetuated by wild carnivores e.g. foxes, Jackals, wolves, mongooses, etc. These wild animals transmit the infection to domestic animals and human through bites.

**Source of Infection**

The saliva of the rabid animals is the main source of infection for humans. The saliva can be infective about three days before the onset of clinical symptoms and during the course of illness till death of the rabid animal.

**Host Factors**

All warm blooded mammals are susceptible to rabies. Humans are an accidental and dead-end host in rabies transmission.

**Mode of Transmission**

Human infection by rabies virus usually occurs as a result of transdermal bite, lick or scratch from an infected animal. Other modes of transmission from animals to humans are possible, for example, when infectious material such as saliva from a rabid animal comes into contact with a victim’s mucous membranes (mouth, nose, eyes) or fresh skin lesions. Ingestion of raw meat or other tissues from animals infected with rabies is not a known source of human infection. Although virus may be secreted in the milk, drinking pasteurized / boiled milk or eating thoroughly cooked animal products do not constitute rabies risk. Skinning or handling of carcasses with bare hands and touching eyes or lips while the hands are contaminated constitute rabies risk.

Transmission through inhalation of virus-containing aerosols have also been reported in the wild in bat populations, however, this mode of transmission is extremely rare. In addition, human-to-human transmission through transplantation of tissue/ organs and two anecdotal cases of transmission through human bite have been reported. Human-to-human transmission of rabies in hospital settings has never been reported.

**Incubation Period**

In humans the incubation period ranges from two weeks to six months but may vary from a few days up to many years. The incubation period depends on the site of the bite, severity of the bite, amount of virus inoculated, species of the biting animal, protection provided by clothing and treatment undertaken. In general the incubation period tends to be shorter in severe exposures and bites on face, head and neck, upper extremities and other highly innervated areas such as fingers since the virus takes lesser time to reach the CNS. The risk is high in the first three months of exposure.
Table 1: Incubation period and duration of illness in animal species and man

<table>
<thead>
<tr>
<th>Species</th>
<th>Incubation period</th>
<th>Duration of illness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog and cats</td>
<td>Average 2-9 weeks</td>
<td>1-10 days</td>
</tr>
<tr>
<td>Cattle</td>
<td>Average 2-15 weeks</td>
<td>(rarely as long as 14 days)</td>
</tr>
<tr>
<td>Sheep and goats</td>
<td>2-17 weeks</td>
<td>5-7 days</td>
</tr>
<tr>
<td>Horses/mules</td>
<td>Average 3-14 wks</td>
<td>2-8 days</td>
</tr>
<tr>
<td>Wild animals</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Human</td>
<td>Avg. 2-24 weeks</td>
<td>2-21 days</td>
</tr>
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</table>

**Pathogenesis**

Rabies virus infects the central nervous system, causing encephalopathy and ultimately death. After inoculation, the rabies virus progresses from the subcutaneous tissue or muscle into peripheral nerves. The virus migrates along nerves to the spinal cord and brain. The victim exhibits behavioral changes and clinical signs when the virus reaches the brain, at this point the incubation period is over and the clinical period begins. The virus ascends into the CNS from the site of bite/exposure rapidly and the spread is marked by progressive encephalitis. After affecting the CNS it spreads from the CNS to the peripheral nerves and salivary glands and rabies may be transmitted to other mammals through infected saliva.

The infected animal usually dies within a week after onset of clinical signs. Factors that may contribute to the development of rabies disease include: the amount of viral inoculums (amount of rabies virus); the anatomic location of the bite or saliva exposure; and post exposure wound management (washing the wound, administering rabies immune globulin and vaccination).

1. **Pathology**

There is no characteristic lesion in the brain except the presence of Negri bodies in the brain cell. Its presence is pathognomonic of rabies infection but absence does not exclude rabies. Vaccination inhibits Negri body formation and this may also be absent if the animal is killed early in the course of the disease.

2. **Viability**

Survival outside host: Rabies virus is inactivated rapidly in sunlight and susceptible to desiccation, however, cold and deep freezing preserves rabies virus.
Disinfectants: Susceptible to all commonly used disinfectant in the health centers such as 1% sodium hypochlorite, 2% glutaraldehyde, 70% ethanol, formaldehyde, and povidone iodine.

Inactivation: Rabies virus is inactivated on exposure to sunlight, pasteurization, boiling, cooking, fermenting, lipid solvents (e.g. soap) and deactivated by 60°C heat for 5 minutes.

Rabies in Dogs

The incubation period in dogs may vary from 10 days to 8 months or even longer. Rabies in dogs may manifest itself in two forms.

1. Furious rabies

These are the typical mad dog syndrome characterized by change in normal behavior:

- Easily irritable
- Bite without any provocation (human, animals, inanimate objects)
- A tendency to eat sticks, mud, straw, etc.
- Tendency to run for no apparent reason
- A change in voice, e.g. barking and growling in a hoarse voice or inability to make a sound
- Excessive salivation or foaming at the angles of the mouth
- Gasping for breath towards the later stages of illness

2. Dumb Rabies

- The dog withdraws itself from being seen or disturbed
- Hanging of jaws & increased salivation
- In this type irritable stage is lacking
- It lapses into a state of sleepiness and
- Dies in about 3 -5 days after it develops clinical symptoms

The characteristic symptoms of hydrophobia are absent in animals.

Rabies in Humans

Early symptoms of rabies in humans are nonspecific, consisting of fever, headache, general malaise, tingling sensation and paraesthesia at the site of the bite. As the disease progresses, neurological symptoms appear and may include insomnia, anxiety, confusion, slight or partial paralysis, excitation, hallucinations, agitation, hypersalivation, difficulty swallowing, and hydrophobia (fear of water). Death usually occurs within few days of the onset of symptoms.
The clinical signs and symptoms include:

- Initially fever, malaise, headache lasting for two to four days
- Pain or itching at the site of the bite wound (in 80% cases)
- Widespread excitation and stimulation of all parts of the nervous systems
- Fear to water (hydrophobia), air (aerophobia), and light (photophobia).
- Increase reflexes and muscle spasm along with dilation of pupil and increased perspiration and lacrimation (motor symptoms)
- Fear of death, anger, irritability and depression (mental symptoms)
- At a later stage the mere sight of water may provoke spasms of the muscles of deglutition.
- The duration of illness is 2 to 3 days but may be prolonged up to 2 weeks
- In paralytic type of rabies, there is ascending type of paralysis (D/D Acute Inflammatory Demyelinating Polyneuropathy e.g. GBS). 
- Death occurs usually due to cardio-respiratory failure as a result of damage to brain stem vital centers.

Standard Case Definition

A subject with history of association or contact with known or suspected rabid animal presenting with acute neurological syndrome (i.e. encephalitis) dominated by forms of hyperactivity (furious rabies) or paralytic syndromes (dumb rabies) progressing rapidly towards coma & death, usually by cardiac or respiratory failure, typically within very short time (7-10 days) after the appearance of signs and symptoms.

Management of Rabies in Humans

Once clinical symptoms of rabies have appeared, rabies is 100% fatal. There is no definitive cure and only symptomatic treatment is possible after symptoms have set in. The treatment should focus on comfort, with heavy sedation (barbiturates, morphine) and avoidance of intubation or life support measures once the diagnosis is certain.

Patient should NEVER be referred to higher centers as handling and transportation exacerbates the symptoms and accelerates death.

Treatment rules include:

- Keep the patient in a quiet room with subdued light and protect from draughts of air or stimuli likely to precipitate spasms and convulsions
- Rabies patients tend to be talkative, avoid disturbing unnecessarily
- Sedation with diazepam 10 mg 4-6 hourly, supplemented by chlorpromazine 50-100 mg if necessary, will help to control muscular spasms and excitability. Phenobarbitones or morphines should be considered if required.

- Feeding orally is usually impossible. Nutrition and fluids should be given intravenously.

**Rabies Vaccines**

The anti-rabies vaccines currently approved by WHO for Intra dermal (ID) use are Purified Chick Embryo Cell Vaccine (PCECV-Rabipur), Purified Vero Cell Vaccine (PVRV-Verorab, Imovax, Rabies Vero, TRC Verorab) and Human Diploid Cell Vaccine (HDCV-Rabivac).

HDCV which is currently available in Bhutan manufactured by Sanofi pasteur are not approved for use by the ID route. It can be only administered through IM route. However, Purified Vero cell Rabies vaccine (PVRV) which can be administered through both intra-dermal (ID) and intra- muscular (IM) route will be procured and supplied in Bhutan.

**1. Pre exposure vaccination**

Pre-exposure rabies vaccination should be offered to high risk group like laboratory staff working with rabies virus and infected material, veterinarians, animal handlers, dog catchers and wildlife workers.

**Pre-exposure ID Regimen: Recommended route of administration when not contraindicated.**

Arrange immunization in a group so that savings on vaccine can be made.

One site 0.1 ml intradermal vaccination on Day 0, 7, 21 or 28 day is given.

**Table 2: Pre-Exposure Prophylaxis using PVRV**

<table>
<thead>
<tr>
<th>Route</th>
<th>Dose</th>
<th>Site</th>
<th>Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra Muscular</td>
<td>0.5ml</td>
<td>One site at deltoid</td>
<td>Day 0, 7, 28</td>
</tr>
<tr>
<td>Intra dermal</td>
<td>0.1ml</td>
<td>One site at deltoid</td>
<td>Day 0, 7, 28</td>
</tr>
</tbody>
</table>
**Booster Dose:**

One site ID vaccination at one year and every 3 years should be given to high risk groups. There should be regular checking of antibody titer among the high risk groups. The rabies antibody titer should be maintained above 0.5IU/ml. If the titer is less than this, a booster dose is warranted.

**Pre-exposure IM regimen:**

When ID vaccinations is not possible or due to immune-compromised status of individual, 0.5ml IM vaccination are given at Day 0, 7, 21 or 28 days. Booster doses of 0.5 ml IM are given at same intervals as per ID regimen. Post – exposure dose of 0.5ml IM are given at Day 0 and 3 with no RIGS like in ID regimen in individuals who have received full pre-exposure regimens and booster doses.

**ID or IM booster vaccination:**

If a person has received ID pre-exposure, he can either get ID or IM booster or Vice versa.

**2. Post-Exposure Prophylaxis (PEP)**

Post-exposure Prophylaxis consists of three procedures:

1. Management of patients following an animal bite
2. Indication for PEP
3. Rabies immunoglobulin and/or anti-rabies vaccine application.

**Management of a patient following an animal bite (First aid treatment)**

- Wounds should be washed immediately with soap under running water for 10-15 minutes.
- Wounds should be cleaned thoroughly at the health care facilities with 70% alcohol or Povidone Iodine.
- Give Tetanus Toxoid (Td) to those who have not been vaccinated (assess vaccination status- DTP or Td in the past).
- Antimicrobials should be prescribed if necessary to prevent bacterial infection.
- AVOID SUTURING THE WOUND. If necessary, this should be done only after infiltration with Rabies Immunoglobulin (RIG).
**Indication for Vaccination and immunoglobulin administration (PEP)**

Factors that should be considered for initiating Post Exposure Prophylaxis:-

- Current outbreak situations among animal
- History of animal bite or other forms of exposure
- Category of exposure (II–III)
- Clinical features in the animal
- Confirmed laboratory test result for rabies

It is essential to assess the rabies risk before the decision is made. A decision tree is presented in Annex 1 and categories of exposure in Table 3.
Table 3: Definition of categories of exposure and use of Post Exposure Prophylaxis (PEP) in case of exposure to suspected or rabid animals

<table>
<thead>
<tr>
<th>Category</th>
<th>Type of Exposure</th>
<th>Risk</th>
<th>Recommended PEP</th>
</tr>
</thead>
</table>
| I        | • Licks on intact skin, touching (petting & bathing), feeding of animals  
          • Consumption of butter, cheese, whey (dachu), curd, buttermilk, and cooked meat  
          • Coming in contact with utensils of a suspected rabid animal on intact skin | None | Not recommended if reliable history is available |
| II       | • Person consuming uncooked meat from rabid animal  
          • un-boiled/un-pasteurized milk  
          • Nibbling of uncovered skin by suspected rabid animal  
          • Minor scratches or abrasions without bleeding  
          • Person who handles, prepares meat or handles the carcass of rabid animals | Minor |  
          • Wound management  
          • Provide ARV immediately |
| III      | • Single or multiple transdermal bites or scratches,  
          • Licks on broken skin,  
          • Contamination of mucous membrane with saliva (i.e. saliva through licks or splash on oral cavity, eyes, nose, external genitalia.) | Severe |  
          • Wound management  
          • Provide ARV immediately  
          • Provide RIG* (only to those with exposure to suspected or confirmed rabid animals) |

Note: It is important to inform the public that milk or meat from suspected or rabid animals must not be consumed.

*Butter is made from curd which is acidic that kills viruses and cheese preparation involves heating which kills viruses.

*Since the antibody level in the vaccinated pet dogs cannot be monitored, vaccinated pets bites are considered similar to stray dog.

*RIG to be provided only for exposure to suspected rabid animal or confirmed rabid animals with ARV but not for all category III exposure to non-rabid animals.
Post-Exposure Vaccination:

Intra-dermal Regimen route using Updated Thai Red Cross regimen–2-2-2-0-2 (Updated TRC regimen) is recommended for administration when not contraindicated.

One dose each (0.1 ml) is given at 2 sites, on both arms (over deltoids) on D0, D3, D7 and D28. (Note: there is No vaccination on Day 14).

Essen Protocol Intra-muscular Regimen

0.5ml of vaccine is administered IM on Day 0,3,7,14 and 28 (Day 0 means day of first dose and not necessarily day of bite). For adults and children aged ≥2 years, the vaccine should always be administered in the deltoid area of the arm; for children aged <2 years, the antero-lateral area of the thigh is recommended. Rabies vaccine should not be administered in the gluteal area, as the induction of an adequate immune response may be less reliable.

Post Exposure Vaccination for Health personnel and relatives who come in contact with human Rabies case

Post-exposure prophylaxis should be provided for health care personnel and relatives considered to be at risk after careful assessment (As per Table no. 2). It may sometimes be necessary to immunize the partners of patients, as close contact and sexual intercourse in the early stages of the disease carry a risk for transmission.

Re-Exposure prophylaxis for previously vaccinated individuals

For rabies-exposed patients who have document of previously completed pre-exposure vaccination or post-exposure prophylaxis, 1 dose should be delivered intramuscularly or intradermally on days 0 and 3. Rabies immunoglobulin is not indicated in such cases. *This is also recommended only if the person has more than 0.5IU/ml of antibody titre, otherwise, he/she should receive full course of PEP.*

In case of incomplete prophylaxis, treat case as fresh exposure and provide PEP and RIGs as per the category.
**Contraindication for ID route**

Patients who are immune-compromised need to be given intra-muscular regimen of ARV as they have poor response to intra-dermal vaccination due to low immunity (e.g. HIV/AIDS, Chronic kidney disease, Cirrhosis of liver, Diabetes Mellitus, R heumatoid arthritis on immune suppression treatment, old age (more than 65 yrs), cancer, organ transplant recipients, Asplenic patients, patients on long term steroid treatment any chronic debilitating illness etc).

**Important points to be noted**

- The dose of tissue culture rabies vaccine is same for children and adult.
- DO NOT administer vaccine in the gluteal region (buttocks).
- Laboratory confirmation of rabies should always be encouraged.
- Patients must be advised not to rub the site of injection after administration of vaccine.
- Patients must be advised to complete full course of vaccine as per the advised schedule.
- All patients who receive rabies PEP should be given a document/card, clearly stating the date, month & year of vaccination and the type of vaccine used.
- D0 means 1st day of vaccination & not day of exposure.

**Rabies immunoglobulin and/or anti-rabies vaccine administration**

All patients in category III with exposure to suspected rabid or confirmed rabid animals and when the status of biting animal is not known (e.g. stray dog, wild animals, etc.) should be given rabies immunoglobulin followed by a course of anti-rabies vaccination. RIGs need not be given for accidental bites by pets which have a history of vaccination and are available for observation.

Category II exposure patients who are Immune-compromised (e.g. patients on chemotherapy, leukemic patients, Kidney transplant recipients, patients on immune-suppressants etc.) should also receive RIGs followed by a course of ARV.

The rabies immunoglobulin provides passive immunity in the form of readymade anti-rabies antibody to tide over the initial phase of infection. It binds with the virus and neutralizes it. Two types of RIGs are available:

**Equine Rabies immunoglobulin (ERIG)**

It is obtained from horses by the process of hyper-immunization. Currently available ERIGs are highly purified and the occurrences of adverse events are minimal. Sometimes ERIG can cause anaphylaxis, however it is cheaper than HRIG.
Human Rabies immunoglobulin (HRIG)

They are literally free from side effects that are encountered with ERIGs. They have longer half-life and require half the dose of ERIGs.

RIG is administered only once at the time of administration of post-exposure prophylaxis. It is used to provide immediate antibodies until the patient’s own immune system responds to active immunization. Rabies immunoglobulin should be administered immediately after the incident with ARV (As per Table no. 2).

Note: If the need for RIG is decided after the vaccination has already been started, it must be administered by 3rd dose (i.e. 7day) and should not be given after the elapse of 7 days as it will counteract with the production of antibody by the body immune system as a result of vaccination.

Dose calculation for Rabies Immunoglobulin:

- Human rabies immunoglobulin (HRIG) - 20 IU/kg body weight (maximum 1500 IU). HRIG preparation is available in concentration of 150 IU per ml
- Equine Rabies Immuno-globulins (ERIG) - 40 IU/kg body weight (maximum of 3000 IU).

Administration of RIGs:

RIG should be infiltrated as much as possible in and around all wounds. After infiltration of the wounds if there is any remaining, it should be given intramuscularly (IM) on the antero-lateral region or deltoid region (away from the site of vaccine administration). Anti-rabies vaccines should be administered preferably on the same day after RIG, but at a different site (e.g. right arm for vaccine and left arm for serum or vice-versa).

Precautions to be taken while administering RIGs

- All emergency drugs and facilities for managing any adverse reactions must be available.
- The RIG vial(s) taken out from refrigerator should be kept outside for a few minutes before administration to the patient (to warm it to room/body temperature).
- RIG should be administered before starting anti-rabies vaccination.
- RIG should not be administered in the same syringe as the vaccine or at the same site as vaccine.
- Pregnancy is not a contra-indication for RIG and anti-rabies vaccination when indicated.
- The patient should not be on an empty stomach.
While infiltrating RIG into bite wounds, care must be taken to avoid injecting into blood vessels and nerves while injecting into finger tips, care must be taken to avoid compartment syndrome.

Anatomical feasibility must always be kept in mind while injecting RIG.

In small children/patient with multiple bites, if the volume is insufficient for infiltration in and around all wounds, dilute RIG with sterile Normal saline up to double or 3 times.

No Live vaccine (OPV, MR, BCG) should be administered for the next three months after the administration of RIGs.

Management of Adverse Reactions

Adverse events following immunization (AEFI)

In general, rabies vaccine and RIGs have been shown to be safe and well tolerated. However, in 35–45% of vaccines, minor and transient erythema, pain and/or swelling may occur at the site of injection, particularly following intradermal administration of a booster. Mild systemic adverse events following immunization (AEFI), such as transient fever, headache, dizziness and gastrointestinal symptoms, have been observed in 5–15% of vaccines. Serious AEFIs like allergic or neurological nature may rarely occur.

Once initiated, rabies prophylaxis should not be interrupted or discontinued because of local or mild systemic adverse reactions to rabies vaccine. Usually, such reactions can be successfully managed with anti-inflammatory and antipyretic agents, such as ibuprofen or acetaminophen.

When a person with a history of serious hypersensitivity to rabies is revaccinated, antihistamines can be administered. Epinephrine should be readily available to counteract anaphylactic reactions, and the person should be observed carefully for at least half an hour after vaccination.

Vaccine storage and transportation

Maintenance of cold chain is of utmost importance to ensure that potency of anti-rabies vaccines is retained. If great care is taken with aseptic technique, an appropriate dose of vaccine may be withdrawn from a vial and the remainder used for another patient, provided that the vial is kept cool and stored in a refrigerator at 2-8°C. A sterile needle and syringe must be used to draw up vaccine for each patient, to prevent cross-infection of hepatitis, HIV and other infections. Although the vaccine antigen is very stable at 4°C, there is a high risk of contamination of multi-dose vials by microorganisms, especially if the vaccine does not contain a preservative. Reconstituted vaccines should be used as soon as possible and those without preservative should be used within 6 to 8 hours if kept at 2-8oC. All unused reconstituted vaccine at the end of 6-8 hours must be discarded.
Laboratory diagnosis of Rabies in humans

Though clinical diagnosis of rabies in humans can be made by the signs and symptoms, the definitive diagnosis of rabies can only be obtained by laboratory investigations.

Diagnosis of paralytic rabies in humans presents a diagnostic challenge. The clinician must have a high index of suspicion of rabies in cases presenting with ascending paralysis symptoms and signs. The differential diagnosis of paralytic rabies are AIDP (acute inflammatory demyelinating polyneuropathy e.g. GBS), ADEM (acute demyelinating polyneuropathy).

Proper sample collection, preservation and transportation are important for laboratory diagnosis; the following samples can be collected and sent for laboratory diagnosis at the earliest possible in cold box (2-4°C);

1. Brain material (Hypothalamus): To be collected aseptically and preserved in a vial containing 50% glycerine saline for virus isolation, fluorescent antibody test and RT-PCR for postmortem diagnosis.

1. Saliva: To be collected aseptically for PCR and rapid diagnostic test for ante-mortem diagnosis.

1. Cerebrospinal fluid: To be collected aseptically by lumbar puncture for ante-mortem or postmortem diagnosis. Usually no preservative is used.

1. Corneal impression, nuchal biopsy: Preserve in vial containing 50% glycerine saline for postmortem diagnosis.

The diagnosis of rabies in men is made by a history of dog bite and manifestations of the typical signs and symptoms. Though ante mortem as well as post-mortem laboratory techniques are available for diagnosis of human rabies, the former is highly insensitive (< 25%), even in the best of hands.

The commonly used laboratory diagnostic methods are briefly described in Table 4.
Table 4: Common laboratory tests used for rabies diagnosis

<table>
<thead>
<tr>
<th>Laboratory Test</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sellers staining for inclusion bodies (Negri bodies)</td>
<td>Simple</td>
<td>Positive in 50 – 70% of cases (obsolete test and not commonly used now)</td>
</tr>
<tr>
<td></td>
<td>Rapide</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Easy to perform</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No special equipment required</td>
<td></td>
</tr>
<tr>
<td>Fluorescent Antibody Test (FAT) for antigen detection</td>
<td>Specific</td>
<td>Expensive</td>
</tr>
<tr>
<td></td>
<td>Sensitive</td>
<td>Requires trained manpower</td>
</tr>
<tr>
<td></td>
<td>Rapide</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Easy to perform</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gold standard test</td>
<td></td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Ante-mortem and post mortem diagnosis.</td>
<td>Requires good training</td>
</tr>
<tr>
<td></td>
<td>Saliva, CSF, brain materials, skin and hair follicles may be used</td>
<td>High risk of contamination in all steps</td>
</tr>
<tr>
<td>Mouse inoculation Test (MIT) for virus Isolation</td>
<td>Can detect very small quantity of virus</td>
<td>Takes long time</td>
</tr>
<tr>
<td></td>
<td>Confirmatory test</td>
<td>Use of laboratory animal (mice)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethical issues involved in use of laboratory animals</td>
</tr>
<tr>
<td>Virus Isolation in Tissue Culture</td>
<td>Rapid</td>
<td>Special cell culture required</td>
</tr>
<tr>
<td></td>
<td>Sensitive</td>
<td>Expensive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trained manpower needed</td>
</tr>
</tbody>
</table>

NOTE BELOW:

Of all the above available diagnostic tests, FAT and RT-PCR are currently used in the National Center for Animal Health (NCAH), Serbithang, Thimphu.

Samples from any suspected cases from health centers may be sent to NCAH at Serbithang, Thimphu for diagnostic support as Clinical laboratories do not have diagnostic services. Please contact 02-351083 or hot line # 124 for more information and support on sample collection and dispatch.
**Documentation**

A standard format should be used for recording and reporting of PEP and rabies cases. The reporting format is presented in Annex II. It will help to analyze rabies situation and provide technical guidance for formulating national policy for rabies control based on changing situation and arranging medical supplies. Rabies data entry into One Health HUBNET is recommended. All patients coming for immunization against Rabies should be issued with Anti Rabies Vaccination card as given in Annex III.

**Investigation of suspected rabies exposures in Humans**

The main objective of investigation of suspected Rabies exposures in humans is to prevent and control further exposures by identifying and controlling the source of introduction, laboratory confirmation of the suspected source implicated, assist in contact tracing of exposed individuals and also to coordinate a multi-sectoral response.

In event of patients reporting to health centers with history of exposure to suspected rabid animals or the products from livestock suspected of Rabies, the form in Annex IV is to be filled in mandatorily and immediately shared with Livestock sector in the locality by the fastest means of communication. The Livestock Centre on receiving the information from their health counterpart in the locality shall form an investigation team involving the relevant official from health sector and carry out joint investigation and advocacy program coordinated by District Livestock Officer. The investigation should include an assessment of the risk of rabies in the animal species involved (including vaccination status, history of potential exposure to other animals of unknown rabies vaccination status, history of potential exposure to livestock products of suspected animals and travel history) and the behavior of the particular domestic animal implicated as per the SOP Annex VIII.

Any information on the confirmed rabies cases in animals in the locality should be shared at the earliest possible with the Chief Medical Officer/District Health Officer/BHU In-charge (Annex V). This will ensure that any person at high risk of rabies after exposure to such animals receive PEP.
1. Department of Livestock

Department of Livestock implements Catch Neuter Vaccinate and Release (CNVR) program to address the increasing number of street-dogs, prevent rabies and other related zoonoses. The CNVR program aims to sterilize and vaccinate 75% of the dog population in the country. CNVR program has sterilized and vaccinated approximately 51000 stray and pet dogs till 2014 nation-wide. The Department also implements registration and vaccination of pet dogs. Rabies awareness, Anti-rabies vaccination campaigns and education programs are coordinated and organized by Department of Livestock and Department of Public Health involving relevant stakeholders such as schools, local governments, in high risk areas annually.

At the geog/district level, the LEC/RNR-EC/DVH is the first focal point of official contact and flash report of the suspected cases are sent to DLO/RLDC/ SVL/NCAH.

At the regional level, the RLDCs will be responsible for epidemiological investigation and laboratory confirmation of the disease, analysis of the data, monitoring of prevention and control activities and feedback of information to the Dzongkhag/geog level and reporting to the National Centre for Animal Health (NCAH).

The NCAH will be responsible for analysis of data at the national level, to study the epidemiological links, trends and achievement of control targets,

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**Reporting and information sharing system**

Accurate and timely information and reporting is necessary to guide human PEP and RIG decisions, determine the management of potentially exposed animals and also to describe the epidemiology of the disease.
formulate national policies and allocate resources and provide advanced laboratory diagnostic support to RLDCs and Dzongkhags. The NCAH shall share with MoH updates on the epidemiological trend of Rabies in animals and also the rabies risk based classification of the country into high risk, medium risk and low risk areas as and when the risk profile changes from the current risk situation.

2. Department of Public Health

The current reporting system and information flow is from BHU/Hospitals/RH to PHL and to DoPH. The national level disease reporting to the Department and Ministry of Health will also be done as per the existing standard notifiable disease reporting system. The information from the BHU, district and regional level are compiled and analyzed at PHL for further reporting to Department and Ministry including WHO.

In general, the reporting of suspected/confirmed Rabies cases and the flow of information will be done in line with the existing reporting system of the Department of Livestock and Department of Public Health but with enhanced sharing of information horizontally at all levels (between two sectors).
Reference:


Annexure I

**DEcision Tree: Guide to Post-Exposure Prophylaxis**

**Animal Bite**

**Which animal species**

**Domestic (dog, cat, cow, goat, sheep, horse, donkey, pig etc.)**

1. Stray dog & cat - Start PEP immediately and observe the animal for 10 days.
2. Provide RIGs for CAT III exposure (only to those with exposure to suspected or rabid animals and immuno-compromised persons - refer page No 13)
3. Share information and collaborate with livestock using forms attached form in annex IV

**Give Td*/Antibiotics if necessary**

**Wild animals (jackal, wolf, mongoose, wild cat, fox, tiger, wild rodents, monkey, bears, etc)**

1. Compulsory ARV vaccination and +/-RIGs
2. Report to Livestock sector using forms attached form in annex IV

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* Tetanus and Diphtheria

**Note:**

1. During the rabies outbreak period, cases of house rat bites should be provided PEP
2. Avoid suturing the wound if possible
### Annex II

**Anti-Rabies Vaccine (ARV) Data Collection Format for Health Centres (Data Register)**

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Registration No</th>
<th>Name</th>
<th>Age/sex</th>
<th>Occupation</th>
<th>Address</th>
<th>Date of exposure</th>
<th>Site of bite*</th>
<th>Source**</th>
<th>Category of Exposure</th>
<th>*** ID PEP course (date)</th>
<th>RIG given (Y/N)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
</tbody>
</table>

*mention the site of bite (leg, arm, face etc)*

**Specify the source (i.e. bite by dog, cat scratch, dog lick, consumption of raw milk, cared for rabies patient, etc)**

***For IM course highlight in the remarks column and provide the date for Day 14 Arv course in the remarks column***
Annex III

ARV card with TT or Td

Ministry of Health
TT or Td vaccination schedule

Name  -----------------------------------------------CID no----------------------------
Age/Gender----------
Place of issue :---------------- date of issue :----------------
Hospital/BHUs registration No.-----------------------------------------------

<table>
<thead>
<tr>
<th>TT/ Td vaccine</th>
<th>Date of vaccination</th>
<th>Remarks TT or Td</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT or Td</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5&lt;sup&gt;th&lt;/sup&gt; dose</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Anti Rabies Vaccine Card

<table>
<thead>
<tr>
<th>Day</th>
<th>Route of Administration</th>
<th>Date of administration</th>
<th>Due date</th>
<th>Remarks (Pre/ post exposure, booster &amp; re-exposure)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IM</td>
<td>ID</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D3</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>D7</td>
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<tr>
<td>D14</td>
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<td></td>
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<tr>
<td>D28</td>
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</tr>
</tbody>
</table>

1. Those who have completed primary series of DTP vaccine with documentation, should not be given Td vaccine up to the age of 5 year during the time of cut injuries and animal bites.
2. Td vaccine is not required to be given for a minimum of 5 years if he or she has documentation of previous immunization with TT or Td vaccine.

Do not lose this card
Annex IV

Reporting Form from health centers to LEC/DVH for rabies exposures

(For any patients reporting to health centres with history of exposure to suspected rabid animals)

Reporting Health Centre:   Date:  
Dzongkhag:  
Name of reporting person:  
Initial Information by:  
1. Telephone_______  
2. Fax. _________  
   2.Others (Specify) ____________________

Patient Details
Name:    Address:       Contact No.:  

Source of Exposure (Tick)
Own Dog   Own Cat   Stray Dog   Stray Cat
Wild animal: (Specify: )   Others: (Specify: )

No. of people exposed to the same animal:

Place of exposure (address):

*Livestock centre to provide feedback to the health centre (result of test, etc.)  
Own Dog/Cat: Owned by individuals/household but not registered as pet
Annexure - V

Reporting form to health facility for confirmed rabies in animals

Reporting Animal Health Centre: Date of report:

Geog: Dzongkhag:

<table>
<thead>
<tr>
<th>Date of case/outbreak</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of report to LEC/RNR-EC/DVH</td>
<td></td>
</tr>
<tr>
<td>Geographical location of outbreak</td>
<td></td>
</tr>
<tr>
<td>Species of animal affected</td>
<td></td>
</tr>
<tr>
<td>Total no. of cases</td>
<td></td>
</tr>
<tr>
<td>Total no. of deaths</td>
<td></td>
</tr>
<tr>
<td>Probable source of outbreak/infection</td>
<td></td>
</tr>
<tr>
<td>Laboratory confirmation from NCAH</td>
<td></td>
</tr>
<tr>
<td>Control measures undertaken</td>
<td></td>
</tr>
<tr>
<td>No. of people exposed* to the infected animal (provide list separately)</td>
<td></td>
</tr>
</tbody>
</table>

*Exposure to rabid dog bites/livestock products from rabid animals
Annex VI

Precautions in handling of dead bodies

The care of a rabies confirmed case and handling the dead body invariably creates anxiety among medical staff, relatives and the public. Blood does not contain the virus, but it is present in many tissues and fluids, such as those of the central nervous system and salivary glands. It is important to remember that there is no documented human to human transmission of rabies till date and human rabies does not pose any greater risk to health care staff than most bacterial or viral infections if routine precautions are used, especially during intubation and suctioning.

Dead bodies of rabies patients should be treated as infectious and handled as follows:

1. The staff/relatives handling the dead body should wear all necessary PPEs – gloves, apron, goggles and surgical mask. A gumboot may be worn as per the risk of contamination
2. All wounds and cuts on the hands of the handling person should be covered before handling the dead body
3. All relatives of the deceased should be explained about the nature of the infection, risk of infections and briefed on the precautions to be taken
4. The body should be put in a leak proof plastic bag and labeled as INFECTIOUS
5. The body should be minimally handled and all tasks relating to handling of the dead body should be performed together as far as possible
6. Embalming and autopsy of the dead body should be avoided but if really necessary should be done with great care and with appropriate precautions and PPE.
7. Disposal of the body is encouraged at the earliest possible by the respective religious practice but body should be disposed by cremation or burial only.
8. Other tissues and body fluids should be disposed of in the same manner as for other infectious diseases.
9. Strict hand hygiene by using soap and water should be followed at all times
Annex VII

Standard operating procedures for IDRV and RIGs administration

Scope: This procedures for IDRV is applicable to all the Health workers using intradermal rabies vaccination and RIGs administration

Pre-requisites: (Service delivery should be through existing delivery system)

- Injection room
- Toilet with tap water for washing the wound
- Trained Health worker
- Attendant
- Table for examining the patient
- Refrigerator
- Weighing machine
- BP apparatus
- Oxygen cylinder with mask

Emergency Drugs

- Anti-rabies vaccines
- RIGs
- Inj Adrenaline
- Inj Promethazine
- Oral anti-histamines
- Inj Hydrocortisone/Dexamethazone
- Inj Ranitidine
- Surgical spirit
- Povidone Iodine
- Normal Saline
- Glucose Saline
- Tetanus Toxoid (Td)
- Antibiotics, Antipyretics, Analgesics and Anti-Inflammatory drugs

Other Supplies and Consumables

- Cotton
- Adhesive plaster
- Dressing material
- Detergent Soap
- Surgical gloves
- Insulin syringes with 26G needles
- 2 ml and 5ml syringes with 24/23 G needles
- Artery forceps
- Toothed forceps
- Swab holder
• Kidney tray
• Dressing bin
• Waste bin
• Stationeries (as necessary)

Procedures for IDRV- (Using Updated Thai Red cross Regimen (2-2-2-0-2))

• Make the patient to sit comfortably and ensure adequate privacy. Patient must be reassured and their anxiety must be alleviated by briefly explaining the procedure that will be performed.
• Using aseptic techniques, reconstitute the vial of freeze-dried vaccine with the diluents and syringe supplied by the manufacturer.
• Roll the reconstituted vaccine vials between the hand and not shake
• Draw 0.2ml of reconstituted vaccine using 1ml insulin syringe (upto 20 units if using 100u or 8units if 40u syringe)
(For pre-exposure and re-exposure vaccination, draw only 0.1ml of reconstituted vaccine from the vial)
• Remove any air bubbles carefully from the syringe to remove any dead space
• Clean the site and stretch the surface of the skin
• Insert the tip of the needle with bevel upwards and keeping it almost parallel to skin, an inch above the insertion of deltoid
• Inject 4 units (0.1ml) intradermal and form a “bleb” (If needle is correctly placed in the dermis, considerable resistance is felt while injecting the vaccine)
• Do not rub the site of injection
• Inject remaining half (0.1ml) into the opposite deltoid
*If the vaccine is injected subcutaneously, papule is not seen. Then the needle should be withdrawn and re-inject (0.1ml) at the adjacent site once more. Withdraw 0.1ml (4U) of reconstituted vaccine into a new syringe and administer at other site.

Intradermal Injection technique
Procedures for ERIGs

1. All emergency drugs and facilities for managing adverse drug reactions must be available.
2. HRIG must be administered before starting ARVs.
3. HRIG must not be administered in the same syringe or site as vaccine.
4. Patient should not be on an empty stomach
5. HRIG vials taken out of refrigerator must be brought to room temperature by keeping outside for few minutes
6. A skin test must be performed prior to administration of HRIG as per product insert guidelines.
7. Human Rabies Immunoglobulin is administered after skin sensitivity test at a dose of 20 IU/kg body weight (Maximum of 1500 IU). HRIG preparation is available in concentration of 150 IU per ml.
8. As much as possible of the recommended dose should be carefully instilled (using 26G needle) into and around the depth of all wounds as far as anatomically feasible.
9. Any remainder RIG should be injected intramuscularly into thigh region (or away from vaccine site) in a single dose.
10. While injecting into finger tips, care must be taken to avoid compartment syndrome.
11. If the volume of RIGs is not sufficient to infiltrate all wounds, it may be diluted using sterile normal saline to a volume sufficient to infiltrate all wounds. (3 times at the maximum)
12. All unused portion of HRIG should be discarded
13. Keep patients under observation for at least 30 minutes
14. If the rabid animal’s saliva falls into the eyes, RIGs can be instilled as eye drops, after dilution (1:1) with sterile normal saline.
15. Service delivery should be through existing delivery system
Annex VIII

SOP for Rabies Outbreak Investigation

An outbreak investigation is a systematic procedure to help identify causes and source of outbreak with a view to control existing outbreak and prevent possible future ones.

Purpose:
- To identify the causes and source of infection
- To identify measures to prevent further spread of disease
- To control and contain the existing disease outbreak
- To assist in contact tracing of exposed individuals
- To coordinate a multi-sectoral response

Scope:
This SOP outlines the general principles and steps for investigation of rabies outbreak in the field

Users:
- Livestock personnel
- Health personnel
- BAFRA personnel

Team Composition:
1. District Livestock Officer (Team Coordinator)
2. Medical Officer (if technical assistance is required)
3. Veterinary Officer (Team Leader)
4. District Health Officer/Assistant DHO
5. Laboratory Technician (Livestock)
6. Geog LEC In-charge
7. BHU In-charge
8. BAFRA official

Materials & equipments:
1. Disposable Gloves
2. Face mask
3. Outbreak Investigation Form
4. Sampling kit
5. SOP/guideline
6. GPS
Steps for investigation:

I. Pre-investigation:
1. Within 6-12 hours of receiving report of suspected rabies in animal or human, the DLO should coordinate to form an Investigation Team
   a. Bring the team members together
   b. Discuss each member’s roles and responsibilities
2. Arrangement of materials and logistics (Refer material list)
3. Gather preliminary information
   a. Name and address of the patient (if reported in human)
   b. Name and address of farmer and species of animal affected (if reported in animal)
   c. Date and time of report of outbreak/cases
4. Provide information to local authority (Gup, Mangmi/Tshokpa) of Investigation Team’s visit to the outbreak area

II. Field Investigation:
1. Background information to collect:
   a. General information of the affected village (No. of household, No. of household rearing livestock, farming system)
   b. General information on buying and selling of livestock and livestock products
   c. Geographical information such as location (X Y coordinates, altitude, road network, Government offices, frequency of movement of people in and out of the outbreak area)
2. Baseline mortality and clinical signs:
   a. General information of the present disease outbreak such as number of households affected, human and animal population at risk etc
   b. Record of the daily morbidity, mortality and case fatality figures in the farm/village
   c. Record of the detail clinical signs during these periods
3. Bio-security arrangement:
   a. Describe type of animal housing
4. Suspected rabid dog:
   a. Determine the presence of suspected rabid dog in the locality
   b. Assess contact with suspected rabid dog
5. Wild animals:
   a. Determine the presence of any wild animals in the locality
   b. Assess contact with wild animals
6. Laboratory investigation
Laboratory investigation in the field (refer specific SOP for sampling, packaging and transportation to the laboratory and rapid field test)

- Put on minimum PPE items (Gloves, face mask, Apron)
- Collect relevant clinical samples from dead animals (Refer SOP for sample collection)

7. Laboratory diagnosis

Following laboratory tests will be done at different levels:

At the field level

- Carry out rapid antigen detection test using immune-chromatographic assay device (if available) At SVL/RLDC/NCAH for confirmation:
- Conduct fluorescent antibody test to confirm rabies outbreak

8. Characterize the outbreak

a. Establish or verify the outbreak
b. Provisional diagnosis made on clinical signs and epidemiological pattern followed by field test.
c. Interim immediate emergency disease control response should be in place before the confirmatory laboratory diagnosis is made from the reference samples from NCAH, Serbithang

Describe outbreak in terms of time, animal and place.

- When was the index case?
- What is the exact period of outbreak?
- Given the diagnosis what is probable period of exposure?
- What are the geographical distributions of the cases?
- What is the pattern of the cases among different species of animals?
- Source of disease outbreak-forward and back ward contact tracing
- Mode of transmission.
- Whether the outbreak is a common source
- What are the risk factors associated with problem?

Control and Prevention (Refer specific SOPS for disposal, decontamination etc)

- Interim immediate emergency disease control response should be in place before the confirmatory laboratory diagnosis is made

Movement control on animal and animal products should be imposed by BAFRA. Strict surveillance and movement control should be maintained on all other properties within the affected area as determined by the team.
The team should:

- Screen at risk human population who has come in contact with suspected animals for eligibility of PEP (Post Exposure Prophylaxis).
- Dispose the carcass and infected materials (Refer SOP)
- Disinfect infected premises
- Sanitary measures, on all the properties within this zone (refer SOPs for disinfection).

Reporting

- Document the findings (Background; investigation procedures, epidemiological and laboratory findings; economic impact etc.)
- Provide recommendations to all the relevant stakeholders (farmers/ producers; DoL, MOH, BAFRA and other agencies)
- Submit the final report.

Risk communication

- Monitor the infected area/community for follow up and contact tracing
- Carry out joint advocacy campaign involving community leaders. Inform them on DOs and DON’Ts (Refer Annex IX) – this should be done together by livestock and health sectors
- All members present in the advocacy campaign should be registered with details of name, gender, address & signature for informed consent to co-operate and support the control of outbreak
**Rabies Outbreak Investigation Form**

<table>
<thead>
<tr>
<th>Reference No.:</th>
<th>Date:</th>
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<tbody>
<tr>
<td>Owner Details:</td>
<td></td>
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<tr>
<td>Name of the farm:</td>
<td>Name of the farmer:</td>
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<tr>
<td>Contact telephone number:</td>
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<tr>
<td>Address:</td>
<td>Geog:</td>
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<tr>
<td>Village:</td>
<td></td>
</tr>
<tr>
<td>Geo Coordinates</td>
<td>Longitude (E)</td>
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<tr>
<td>Information about the farm</td>
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<tr>
<td>Type of Farm:</td>
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</tr>
<tr>
<td>Commercial</td>
<td>Semi-commercial</td>
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<tr>
<td>Livestock population (<em>mention in detail including sex, breed, age and categories)</em>:</td>
<td></td>
</tr>
</tbody>
</table>

**Detail history of outbreak:**

- Actual location and area (Descriptive geographical information)
- Date and time of report of outbreak from farmer/health sector to LEC/DVH/SVL
- Date and time of report from LEC/DVH/SVL to RLDC/NCAH
- Date and time of onset of clinical signs:
- Date and time of onset of mortality:
- Any outbreak in the past years (mention the details)
- Any meat or milk consumed from the herd or the sick/dead animals (if yes, mention the details)
## Animals affected

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Species</th>
<th>Age &amp; Sex of animals affected</th>
<th>Number of Villages affected</th>
<th>Number of Cases</th>
<th>Number of Deaths</th>
<th>Source of infection</th>
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### Clinical observation

*Detail clinical signs (including duration of illness)*

Comments on epidemiology of the disease (origin of disease, mode of entry, how the disease spread in the population, any human exposures etc)

### Laboratory findings

### Differential diagnosis

### Disease suspected/confirmed

### Control measures recommended

### Sample ID

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Category of livestock</th>
<th>Specimen type</th>
<th>No. of specimens</th>
<th>Laboratory referred to</th>
<th>Date of shipment</th>
<th>Test requested for</th>
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</table>

Any additional information:

Name & Designation of Investigation team:
Annex IX

SOPs /Guidelines for Information Education & Communication at Community Level

1. Information & communication to the gathering regarding rabies should be limited to the contents of the National Guideline for Management of Rabies
2. All the members present during the gathering should be registered with details of name, gender, address and signature for informed consent to cooperate and support the control of existing outbreak.
3. Explain the DOs and DONT’s clearly to the gathering and clarify any doubts the gathering request’s on rabies

4. DO’s

a. In case of animals bites, wash the wounds immediately with soap and running water for 15 minutes
b. Do not milk cows if the animal exhibits signs of rabies
c. Always boil milk for self consumption
d. Tie up / tether all suspected animals. If possible, only one person to handle suspect animals. The person handling suspect animals should have self protection (like gloves, mask etc). Wash hand with soap and water for 15 minutes after handling the animals
e. Bury the animal carcasses with minimal handling, engage minimal persons. Use personal protective equipment and wash hand with soap and water for 15 minutes
f. Report any new suspected case to Livestock officials
g. Complete full course of ARV vaccination once started
h. Inform any suspected case of rabid animals in your locality to the nearest Livestock centers/health centers/BAFRA. Call hotline no. 124/155.

5. DONT’s

a. Do not drink raw/unboiled milk
b. Do not make milk products like butter, cheese, dachu (whey), butter milk from unboiled milk
c. Do not sell milk products
d. Do not consume meat of dead animals without examination by the Livestock/BAFRA officials
e. Do not let healthy cattle/other animals come near the sick ones
Annex X

Standard operating procedure for rabies sampling

Preparation for rabies sampling

1. Technicians collecting brain samples for rabies confirmation should be in appropriate Personal Protective Equipment (Gloves, mask, apron, shoe cover etc.)
2. Appropriate sampling equipments in place

Collection of brain samples

1. Collect entire cerebellum and underlying brain stem in case of large animals (Cattle, horses, etc)
2. Collect hippocampus in case of canines and other small animals (dogs, cats, etc)
3. Preserve half portion of brain in 50% Glycerol saline and other half in 10% formalin.
4. Alternatively, each piece from hippocampus, cerebellum and brain stem in 50% glycerol saline and in 10% formalin.
5. The container should be sealed and packed in thick and hard box labelled “Suspected for rabies”.
6. Fresh smears from the brain may be stained with seller’s stain.
7. Formalin inactivates the virus, thus virus isolation tests cannot be used and diagnosis depends on using a modified direct fluorescent antibody test (FAT), polymerase chain reaction (PCR), (less sensitive than these tests on fresh tissue), immuno-histochemistry or histology (Warner et al., 1997);
8. Infectivity at room temperature may be extended for several days if brain material is kept in a mixture of 50% glycerol in phosphate buffered saline (PBS). Glycerol/PBS slows bacterial action and therefore protects against the chemical and biological effects of putrefaction. It does not protect against titre decline due to thermal conditions and therefore, because rabies is thermo-labile, the virus titre will decline during glycerol/PBS storage. Under normal transport conditions in the tropics, this protection may only be effective for a matter of several days. Therefore, whenever possible samples in glycerol/PBS should be kept refrigerated. As the virus is not inactivated by glycerol/PBS, all laboratory tests can be used on these samples.
9. An alternative for the transport of samples for molecular techniques is the use of FTA Gene Guard system (Picard-Meyer et al., 2007). The FTA paper preserves rabies virus RNA within the fibre matrix allowing the transport of samples at ambient temperature without
specific biohazard precautions for further characterization of rabies strains.

10. Refer these samples for laboratory confirmation to appropriate laboratories for rabies confirmation (RLDCs and NCAH)
Annex XI

Standard Operation procedure for disposal of rabid carcasses by burial

Purpose:
To have standard procedure for safe disposal of rabies infected carcasses

Scope:
This section describes procedures for site selection and burial of rabies carcasses in a safe manner

Users:
Veterinary Officer/Para veterinarians

Manpower:
Veterinary Officer, para-veterinarians, animal owners

Materials/ Equipments required:
Hand gloves; Face masks; Apron (disposable); Eye goggle; Gum boot; Disinfectant-Lime/ Virkon
Digging tools: spades, crowbars, peak-axe

Procedure:
Select an appropriate site for carcass burial

- Due consideration should be given not to contaminate water sources, residential areas, livestock facilities, pastures and other establishments in the vicinity. Preferably it should be away from any footpaths or roads leading to the site.
- Prepare a pit with sufficient width to accommodate the carcasses with a minimum depth of 2 meters considering the size of the carcasses.
- Wear apron, face masks, goggle and gloves before handling the carcasses.
- Drop the carcasses into the pit.
- Cover the carcasses with soil, 400 mm is suggested, and add an unbroken layer of lime (calcium carbonate)
- Do not put lime directly on to the carcasses (it will slow decomposition process).
- Close the pit with sufficient soil and make a heap over the site.
- Then put a layer of lime over the soil
- Secure the disposal site by fencing and place a notification mark.
Annex XII

Standard Operating Procedure for disinfection and decontamination of contaminated premises and materials.

Purpose:
To have standard procedure for effective disinfection and decontamination of contaminated premises and materials

Scope:
The document describes procedures for disinfection and decontamination of contaminated materials and premises.

Users:
Veterinary Officers/Para-veterinarians

Manpower:
Veterinary Officer/para-veterinarians; animal owners

Materials/ Equipments required:
Gloves; Apron; Gum boots; Buckets; Mugs/jugs; Water; bleaching powder; hypochlorite

Procedure:
• Prepare 1% hypochlorite solution in a bucket.
• Utensils: Spray and wash barn utensils, tools and equipments with the above solution thoroughly.
• Dry them for reusing.
• Burry the beddings with carcasses if it is in small quantities/ burn it in a pit if in larger quantities.
• Contaminated premises should be disinfected thoroughly with the 1% hypochlorite spray @ 1-1.5 lts/sq. mts. Allow contact time of 2-3 hrs.
• Contaminated laboratory materials can be disinfected by immersing them in 1% hypochlorite solution for at least 30 minutes. Disposable items, including used PPEs must be incinerated/burnt in a pit